Vol. 93, No. 2 October, 1947

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

PHYSIOLOGY OF INSECT DIAPAUSE. II. INTERACTION BETWEEN THE PUPAL BRAIN AND PROTHORACIC GLANDS IN THE METAMORPHOSIS OF THE GIANT SILKWORM, PLATYSAMIA CECROPIA

CARROLL M. WILLIAMS[1]

The Biological Laboratories, Harvard University, Cambridge, Mass.

In the Cecropia silkworm the progress of metamorphosis is interrupted as soon as the pupa is formed. There then intervenes a prolonged period of pupal diapause characterized by cessation of growth and differentiation. The mechanism that converts this cellular dormancy of diapause into the intense activity of adult formation was examined in a previous investigation performed on several species of giant silkworms (Williams, 1946b). For these species it was evident that diapause is under the control of the insect's brain. This control consists in a dependency of adult development on the action of a factor arising from the brain; the brain, in turn, is rendered competent to release this factor by exposure to low temperatures. It is the purpose of the present report to describe in greater detail the nature of the activating mechanism.

Materials and Methods

The present communication is based on a total of 282 experiments performed, for the most part, on pupae of the giant silkworm, *Platysamia cecropia*. In a few experiments pupae of related genera were studied, including *Telea polyphemus, Callosamia promethea,* and *Samia walkeri*. The management of this array of animals was essentially identical to that described previously (Williams, 1946b).

Direct or Indirect Action of the Brain?

If the brain is removed from a diapausing pupa, the resulting insect never develops further and persists until death in permanent diapause—a matter of two years in some individuals. Yet at any time during this period adult development can be evoked by implanting into the brainless pupa a brain obtained from a previously chilled pupa.

It was a rational assumption that the developmental factor from the implanted brain had some direct action on the host tissues whereby the latter were converted from dormancy to activity. Yet the possibility remained that the brain's action might be indirect rather than direct, in a sense familiar to endocrinologists, as, for

[1] The assistance of the Society of Fellows, Harvard University, and the Lalor Foundation, Wilmington, Delaware, is gratefully acknowledged.

example, in the action of certain of the pituitary hormones on the uterus. Experiments have therefore been performed to test these two possibilities.

To this end techniques have been developed for subdividing individual pupae into fragments. In my experience division of these stout animals by means of ligatures has not met with success. Nor did gross slicing of the pupae into parts, as practiced by Crampton (1899), Hirschler (1903, 1904), and Hachlow (1931), produce viable preparations. A simple, successful technique that was ultimately developed will be described briefly.

Isolation of Pupal Fragments

The pupa, under continuous carbon dioxide anesthesia (Williams, 1946a), is placed on its side and the abdominal cuticle plus underlying hypodermis incised around the circumference of the abdomen at the level of the tips of the wingflaps. The incision is confined to the thin intersegmental membrane. Further maneuvers are designed to separate the abdomen from the anterior fragment without rupturing the midgut.[2] The intersegmental muscle masses, the heart, and the nerve cord are, in turn, cut through. The fat body and hindgut are then transected and the attachments of tracheae swept away from the walls of the midgut. The midgut plus nearly all of the Malpighian tubules may now be placed in the anterior fragment. Or by further dissection the midgut may be removed from the anterior fragment and discarded.

The cut surface of each of the two fragments is then sealed by melted paraffin to a circular, plastic cover slip which is provided with a centrally placed hole. Through the hole insect Ringer's is added to displace all air and the hole is finally plugged with melted paraffin.

It may be noted that these isolated abdomens (Fig. 3) consisted, in reality, of the terminal six abdominal segments, the anterior four abdominal segments being carried away with the anterior end (Fig. 1). Transections at other levels were occasionally accomplished, but the one described proved to be most favorable. In

[2] Rupture of the midgut floods the body cavity with a dark green fluid that contains a good deal of particulate matter and shows a broad, dense absorption band centering at 670 mμ. The fluid is apparently non-toxic, but the particulate matter is frequently drawn into the cut end of the heart. Within the heart it then acts as embolus to occlude the aorta at its narrowest portion just behind the brain. Such animals usually fail to survive.

Explanation of Plate I

Approximately Life Size

Figure 1. Anterior fragment of a brainless, diapausing pupa.

Figure 2. Fragment in Figure 1, after adult formation. Development was evoked by implanting a previously chilled brain.

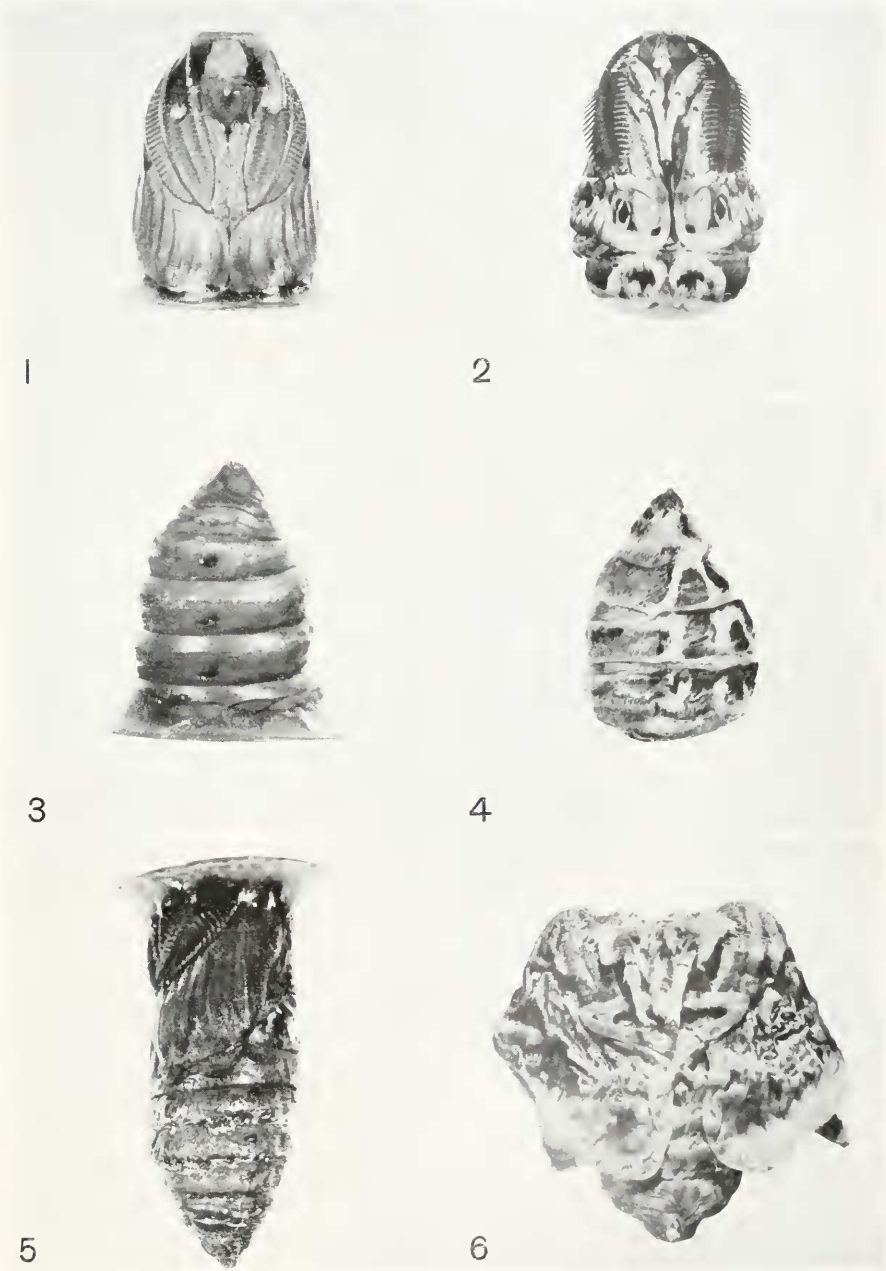
Figure 3. Posterior fragment of a diapausing pupa.

Figure 4. Fragment in Figure 3, after adult formation. Development was evoked by implanting a previously chilled brain plus two pairs of diapausing prothoracic glands.

Figure 5. Posterior fragment of a diapausing pupa, showing critical level of section that includes the meso- and metathorax.

Figure 6. Fragment in Figure 5, after adult formation. Development was evoked by implanting a previously chilled brain. Note the complete development of those parts of the antennae, legs, and wings that were present in the fragment.

PLATE I



certain instances abdomens were transected a second time to yield viable pairs of abdominal segments sandwiched between plastic slips.

The inner surface of the plastic slips is rapidly coated by a thin, transparent tissue formed by anastomoses developed between blood cells and, within about two weeks, by the outgrowth of epithelium and of tracheoles. At any time, however, one can operate inside the insect fragment by removing the paraffin plug from the centrally placed hole.

Of the entire series of isolated pupal parts, approximately two-thirds died within the first ten days after preparation. Death was invariably preceded by a darkening of the blood, a reaction which in itself seems to be toxic. The remaining preparations survived for considerable lengths of time, as indicated by the beating of the heart and by spontaneous movements of the abdominal segments. Isolated abdomens remained alive at 25° C. for up to eight months. Anterior ends survived for not more than two or three months, however, unless Ringer's solution was occasionally added to compensate for loss of water by evaporation.

Brain Implantations Into Pupal Fragments

Brainless diapausing pupae were transected at the level of the wing-tips to obtain ten pairs of viable anterior and posterior halves. Into each of these subdivisions a chilled Cecropia brain was then implanted (Fig. 1 and 3). Each anterior fragment proceeded to develop normally into the corresponding anterior end of a lively adult moth, the cut surface being closed by scaleless, regenerate, "chitinized" epithelium (Fig. 2). The isolated abdomens, to the contrary, remained undeveloped after brain implantation, although they continued to live for an average of three months.

With slight variations in technique these findings have been confirmed during the past three years on a total of 60 additional viable preparations. As many as six chilled brains have been implanted into a single abdomen without inducing development, even though some of these abdomens survived as long as eight months thereafter.

Explanation of Plate II

Approximately Life Size

Figure 7. Posterior fragment of a diapausing pupa joined directly to the tip of a brainless, diapausing pupa.

Figure 8. Preparation in Figure 7, after adult formation of host and graft. Development was evoked by implanting a previously chilled brain into the host. Note the regenerate tissue passing through the hole in the plastic slip to connect the two parts.

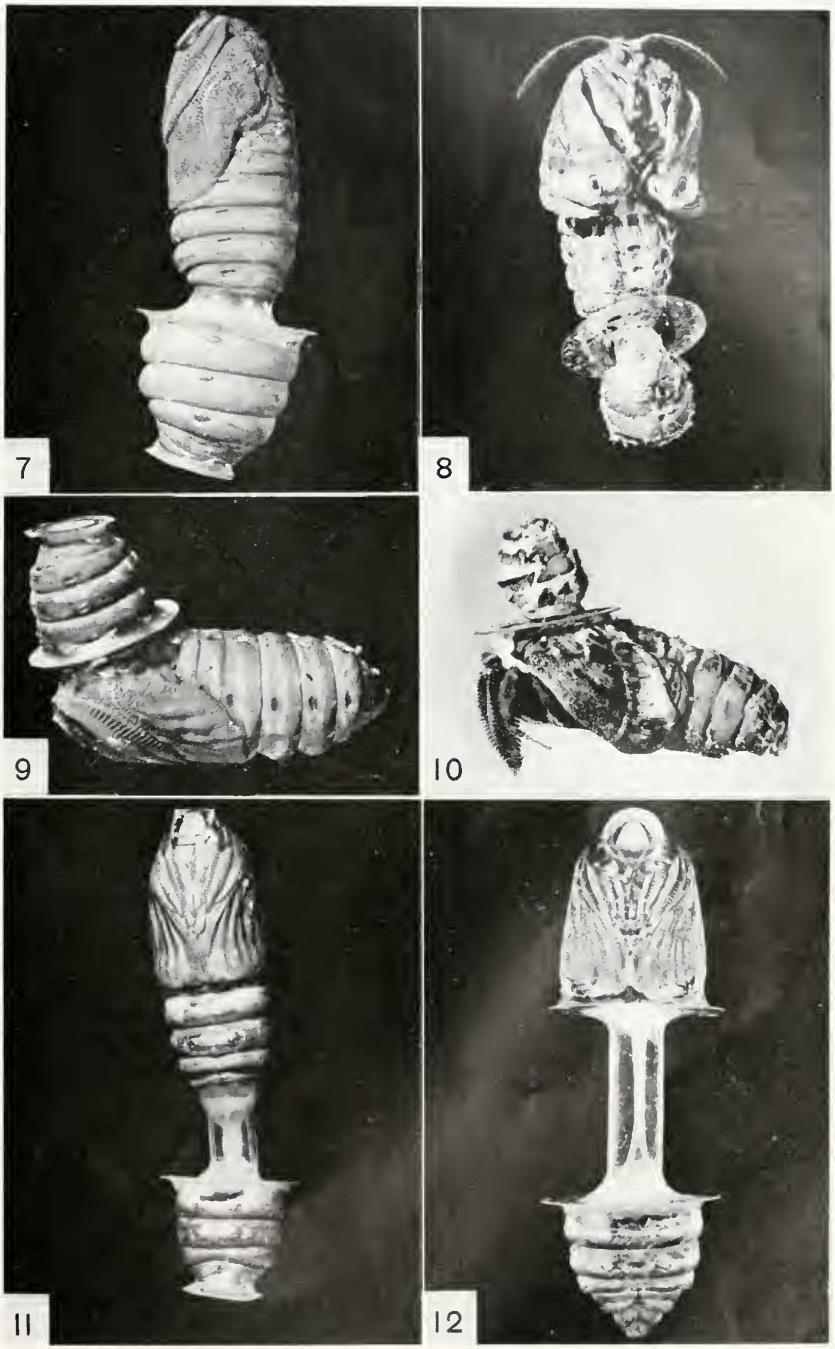
Figure 9. Posterior fragment of a diapausing pupa joined directly to the thoracic tergum of a brainless, diapausing pupa.

Figure 10. Preparation in Figure 9, after adult formation of host and graft. Development was evoked by implanting a previously chilled brain into the tip of the graft.

Figure 11. Posterior fragment of a diapausing pupa joined to a brainless, diapausing pupa by means of a short, square, plastic tube. Note the tube of regenerate tissue traversing the plastic tube's lumen. In order to follow the onset and progress of development, plastic windows have been placed at each end of the preparation.

Figure 12. Posterior fragment of a diapausing pupa joined to an anterior fragment of a brainless, diapausing pupa by means of a long, plastic tube. Note the tube of regenerate tissue within the plastic tube.

PLATE II



It is therefore apparent that anterior and posterior fragments respond differently to brain implantation. This difference in response might be explained if the anterior fragment possessed a second developmental center that was lacking in the posterior fragment. To test this possibility the level of transection was varied.

Transections of Pupae at Various Levels

Brainless, diapausing pupae were transected at various levels and the developmental capacity of the posterior fragments tested by implanting a chilled brain. The results of these experiments confirmed the findings of previous investigators that a "differentiation center" was present in the region of the thorax of lepidopterous pupae (Hachlow, 1931; Bodenstein, 1938; Bounhiol, 1938). Thus, abdomens in continuity with a pupal thorax invariably developed when a chilled brain was implanted. In contrast, similar treatment failed to evoke development of abdomens separated from the entire thorax. The critical level seemed to lie in the region of the mesothorax, for, after transection at this point, brain implantation induced development only after a long latent period of about six months (Fig. 5 and 6).

Grafting Experiments on Isolated Abdomens

The importance of this anterior differentiation center was further revealed in experiments in which isolated abdomens were grafted to brainless, diapausing pupae (Fig. 7, 9, and 11) or to the anterior fragment of brainless, diapausing pupae (Fig. 12). In such combinations, the host and the graft grew together to establish tissue continuity, but did not develop further. This continuity was developed even when plastic tubes, as long as 3 cm., were interposed between the two parts (Fig. 11 and 12).

Now, when a brain from a chilled Cecropia pupa was implanted into either host or graft, diapause was terminated and the entire preparation developed into the fully formed, corresponding parts of the adult (Fig. 8 and 10).

Implantation of Prothoracic Glands

The evidence, up to this point, indicates that adult development after pupal diapause requires a factor from the brain plus some additional factor from the thorax. An extensive series of experiments was then performed in which a chilled brain plus various thoracic organs were implanted into isolated abdomens.

This search was vastly aided by the publication of Fukuda's paper in 1941. In this communication Fukuda describes an endocrine activity of the "prothoracic glands" in evoking development of ligatured pupal abdomens of the commercial silkworm, *Bombyx mori*.

The great significance of the prothoracic glands was confirmed. Thus, isolated abdomens of diapausing Cecropia pupae developed readily when provided with a chilled brain plus two pairs of prothoracic glands.[3] The pupal cuticle became crisp

[3] Due to the numerous ramifications of the pupal prothoracic glands in the pro- and mesothorax, it was usually impossible to remove these organs in their entirety. For this reason two pairs were usually implanted into each isolated abdomen. This proved to be an important detail, for a single pair of the incomplete glands did not generally suffice to produce development after brain implantation. For a description of the prothoracic glands in *Platysamia cecropia* see Williams, 1948.

and was delaminated and the abdomens appeared as the lively, corresponding segments of the adult, the cut surface being closed by scaleless, regenerate, "chitinized" epithelium (Fig. 4). Development was complete externally and internally and, in the case of female abdomens, the eggs were matured. Similarly, abdomens isolated from chilled pupae after return to room temperature required both a chilled brain and prothoracic glands for the initiation of adult development.

In these experiments it was found that the prothoracic glands as well as the brain show a lack of species—or genus—specificity, for both of these organs remain effective when interchanged between *Platysamia cecropia* and *Telea polyphemus*.

In contrast to the results of Fukuda (1941), who studied a species without pupal diapause, no development of isolated abdomens occurred when prothoracic glands were implanted in the absence of a chilled brain.

Further Experiments on Isolated Pupal Abdomens

From a series of six isolated abdomens of diapausing Cecropia pupae there were further removed the gonads, the entire digestive tract, the Malpighian tubules (with the exception of a few loose fragments), and the entire central nervous system, including the residual chain of five ganglia and connectives. Three of these preparations survived, the heart continuing to beat. After the implantation of a chilled brain plus prothoracic glands, two of the abdomens developed into flaccid, but fully mature adult abdomens.

Discussion

It is evident from these experiments that the termination of pupal diapause requires a minimum of two factors, one from the brain and the other from the prothoracic glands. The easiest way to render these organs functional is to expose the diapausing pupa to low temperatures and then return it to room temperature. As has been shown previously, the effectiveness of chilling in terminating dormancy can be explained in terms of an action of low temperature on the brain, whereby the latter is made competent to release its developmental factor (Williams, 1946b). Manifestly, the prothoracic glands do not require similar exposure to cold, for they are promptly activated when a chilled brain is implanted into a diapausing pupa.

From these observations it may be concluded that the brain exerts a controlling action on the prothoracic glands. Subsequent experiments have confirmed this hypothesis consistently. Thus to induce the development of isolated pupal abdomens, it is necessary that the implanted brain be obtained from a previously chilled pupa. This is not the case in regard to the implants of prothoracic glands, for these organs are equally effective when obtained from diapausing pupae. The functional failure of the prothoracic glands at the outset of diapause seems therefore to result from a primary failure of the brain in releasing its factor.

The mechanism that terminates diapause must ultimately supply the dormant tissues with something necessary for cellular growth and differentiation. In respect to this process the experimental results may be interpreted from two points of view. On the one hand, the brain factor may be conceived as having sole action on the prothoracic glands and the prothoracic gland factor, in turn, as having

specific effect on the tissues of the body in general. On the other hand, the tissues may require interaction with both factors, the first factor serving to condition the tissues for reaction with the second.

Although the evidence at hand does not suffice to exclude either interpretation,[4] it seems likely that the factor from the prothoracic glands has ultimate action on the dormant tissues to convert them to activity. Thus, we have noted previously that adult formation becomes independent of brain action as soon as the earliest

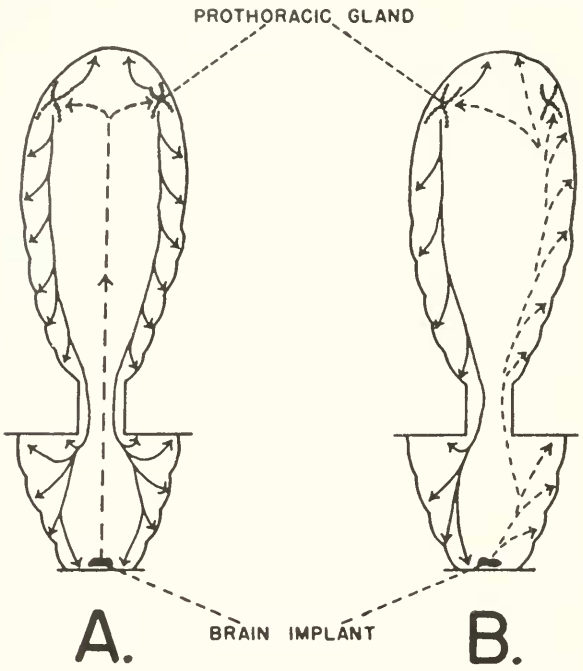


FIGURE 13. Diagrammatic interpretation of experimental results when a chilled brain is implanted into the tip of the graft in preparations such as Figure 11. Development begins first in the anterior end of the pupa, containing the prothoracic glands. For explanation, see text.

sign of adult development is evident (Williams, 1946b). However, for some days thereafter, the animal continues to require further function of its prothoracic glands, for if the abdomen is isolated during this period, it ceases to develop. When tested in this fashion, adult development shows a dependency on the prothoracic glands until a critical stage, signalled by the initiation of eye pigmentation.

Further evidence favoring the view that the factor from prothoracic glands has ultimate action on the tissues has been derived from the experiments, described above, in which isolated abdomens were grafted to brainless, diapausing pupae (Fig. 7 and 9). When development was evoked by implanting a chilled brain *into the tip of the graft,* the brainless pupa, containing the prothoracic glands, was

[4] Since this paper went to press, proof has been obtained of the validity of the first interpretation (see Fig. 13*a*).

observed to initiate development one day in advance of the abdomen, containing the brain. This difference in time can be magnified to three days or longer by interposing a plastic tube between host and graft (Fig. 11). In such preparations the final organ to initiate development was the imaginal disc of the graft's genitalia, notwithstanding the fact that this lay alongside the implanted brain that had touched off the whole process.

In terms of this type of preparation we may finally summarize our present information diagrammatically, as indicated in Figure 13. In Figure 13*a*, the brain factor is viewed as activating the prothoracic glands and the prothoracic glands as activating the tissues. In Figure 13*b*, the brain factor is conceived to act on all the bodily tissues to prepare them for final reaction with the factor from the prothoracic glands. In either case, the brain is the organ of primary control, but this control, at least in part, is exercised by an indirect mechanism.

Summary

1. The mechanism that initiates adult development after pupal diapause has been studied in a total of 282 experiments, supplementary to those reported previously.

2. Brainless, diapausing pupae were divided transversely and the developmental capacities of anterior and posterior fragments tested and compared.

3. Implantation of a previously chilled brain sufficed to terminate the dormancy of anterior fragments.

4. Isolated abdomens, to the contrary, remained undeveloped after brain implantation. Yet such abdomens, even without implantation, could be induced to develop by grafting them to developing anterior fragments. Manifestly, the abdomens required for development an additional factor normally produced in the anterior end of the pupa.

5. By transections at various levels the source of this additional factor was found to be the thorax.

6. A testing of various thoracic organs revealed the effectiveness of the "prothoracic glands." Thus, implantation of a chilled brain plus prothoracic glands induced the complete adult development of isolated abdomens. In this effect the prothoracic glands as well as the brain showed a lack of species—or genus—specificity.

7. The termination of diapause requires in these species the action of a minimum of two factors, one arising from the brain and the other from the prothoracic glands. The brain factor is necessary for the activation of the prothoracic glands.

8. The factor from the prothoracic glands, most probably, has ultimate action on the tissues in terminating diapause.

9. The brain is the organ of primary control over diapause in the species studied, but this control, at least in part, is exercised by an indirect mechanism.

Literature Cited

Bodenstein, D., 1938. Untersuchungen zum Metamorphoseproblem. II. Entwicklungsrelationen in verschmolzenen Puppenteilen. *Arch. f. Entwmech. d. Organ.*, **137**: 636–660.

Bounhiol, J. J., 1938. Recherches expérimentales sur le déterminisme de la métamorphose chez les Lépidoptères. *Bull. Biol. de France et de Belgique, Suppl.*, **24**: 1–199.

CRAMPTON, H. E., 1899. An experimental study upon Lepidoptera. *Arch. f. Entwmech. d. Organ.*, **9**: 293–318.

FUKUDA, S., 1941. Role of the prothoracic gland in differentiation of the imaginal characters in the silkworm pupa. *Annot. Zool. Japan.*, **20**: 9–13.

HACHLOW, V., 1931. Zur Entwicklungsmechanik der Schmetterlinge. *Arch. f. Entwmech. d. Organ.*, **125**: 26–49.

HIRSCHLER, J., 1903. Studien über Regenerationsvorgänge bei Lepidopteren-Puppen. *Anat. Anz.*, **23**: 612–627.

HIRSCHLER, J., 1904. Weitere Regenerationsstudien an Lepidopterenpuppen (Regeneration des vorderen Körperendes). *Anat. Anz.*, **25**: 417–435.

WILLIAMS, C. M., 1946a. Continuous anesthesia for insects. *Science*, **103**: 57.

WILLIAMS, C. M., 1946b. Physiology of insect diapause: the role of the brain in the production and termination of pupal dormancy in the giant silkworm, Platysamia cecropia. *Biol. Bull.*, **90**: 234–243.

WILLIAMS, C. M., 1948. Structure and function of the prothoracic gland in the metamorphosis of the Cecropia silkworm. (In Preparation.)